ON THE PRESENCE OF HEMOLYTIC SUBSTANCES IN EDIBLE FUNGI.*

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IT has recently been pointed out by Abel and Ford[1] in a paper dealing with a chemical analysis of the constituents of *Amanita phalloides* that two poisons are present in this fungus, and that they may be separated by precipitation with ethyl alcohol. The alcohol precipitate contains the hemolytic substance originally described by Kobert[2] under the name "phallin," while the alcohol filtrate contains the Amanita-toxin found in the "deadly Amanita" by Ford[3] which is possibly but not certainly identical with a poisonous substance described by Kobert[4] and which he believed to be an alkaloid. It was also shown by Abel and Ford[5] that this hemolytic substance is not a "toxalbumin," as stated by Kobert, but a glucoside extremely sensitive to the action of heat, acids, and the digestive ferments. From these properties the authors conclude that this hemolytic glucoside can play no rôle whatever in intoxications by *Amanita phalloides* in man, since in these cases the cooked fungi are introduced into the stomach as food. The active principle is therefore the heat-resistant Amanita-toxin contained in the alcohol filtrate. It had already been suggested by Kunkel[6] on the basis of his pupil Seibert's[7] work, that phallin could not be the poisonous constituent since it was absent from dried specimens of *Amanita citrina*, a toxic species either closely related to or identical with *Amanita phalloides*. Finally Kobert[8] has recently receded from his earlier position in the matter, stating

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[1] *Jour. Biol. Chem.*, 1907, 2, p. 273.

[2] *St. Petersburger med. Wchnschr.*, 1891, 16, pp. 463, 471.

[3] *Jour. Exper. Med.*, 1906, 8, p. 437.

Sitzungsber. d. naturf. Gesellsch. zu Rostock, 1899; also *Lehrbuch der Intoxicationen*, Stuttgart. 1906, 2, p. 625.

[5] *Loc. cit.*

[6] *Handbuch der Toxicologie*, 1901, 2, p. 1048.

[7] *Inaug. Dissert.*, München, 1903.

[8] *Lehrbuch der Intoxicationen*, 1906, 2, p. 763.

that phallin is by no means always present in the fungus, the active principle of which he thinks is still a matter for investigation.

In view of these various opinions it became a point of importance to study fresh specimens of *Amanita phalloides*, to determine whether hemolysins are always present in them and whether they disappear on drying, and finally to ascertain if similar blood-laking substances may be found in other fungi. This work was undertaken during the summer of 1906 at the Marine Biological Laboratory at Woods Hole, Mass., and I should like here to express my indebtedness to the acting director, Dr. Frank Lillie, and to the botanist, Dr. Moore, for many courtesies extended to me during the progress of the investigation. The pine woods in the vicinity of Woods Hole and on the adjacent islands abound in fungi of all sorts and descriptions, and an excellent opportunity was thus presented for studying perfectly fresh specimens. A large amount of material was also collected and dried, a subsequent study of this material being made in the Bacteriological Laboratory of Johns Hopkins University. The most important facts brought out during this study relate to *Amanita phalloides*, and to two closely related species of Amanita, *Amanita rubescens* and *Amanita solitaria.*

AMANITA PHALLOIDES.

Typical specimens of *Amanita phalloides* are in many cases quite devoid of hemolytic activity. A large number of individual plants were tested upon rabbits' and hens' blood corpuscles, both of which are sensitive to the action of the hemolysins in this fungus, and in many instances no laking of the corpuscles could be demonstrated. In other plants hemolysins were found in their usual strength, the distribution of these substances being seemingly independent of the locality in which the fungi were collected. In agreement with Kobert,[1] therefore, we must look upon this hemolytic substance as by no means constantly present in the "deadly Amanita," and on this account alone it could not be considered the active principle. Still more important facts were brought to light by the study of two other fungi, both of which are considered edible, one of which is powerfully, the other moderately, hemolytic.

[1] *Loc. cit.*

AMANITA RUBESCENS.

This is a very large Amanita which was especially abundant during the wet season in July. In appearance it is a typical Amanita. It has a widely expanded pileus of a dull-red color, covered, especially in the early stages, with thick, yellowish scales. The under surface of the pileus, the spores, the gills, and the stalk, are pure white, as well as the veil and poison cup.

As soon as the plant is touched or handled, however, the bruised flesh assumes a dull-red color, and the coloration is the most important means of recognizing the species. The change in color occurs within a few minutes, and by the time the plant has been taken to the laboratory but little of the original white is seen. The juice from the macerated fungus is of a dull-red color, while the dried specimens have this color markedly accentuated. Only when the plants are very young do they retain their typical appearance. The *Amanita rubescens* is considered edible by the majority of mycologists, Atkinson, and others, the only objection to its use for the table being its resemblance to *Amanita phalloides*. As far as the literature shows it is never the cause of poisoning.

When tested upon rabbits' and hens' corpuscles the juice of *Amanita rubescens* is powerfully hemolytic. Solution of the corpuscles takes place rapidly within 15 minutes to an hour, and in a far greater dilution than in any specimen of *Amanita phalloides* I have ever seen. The action of the hemolysins is similar to that of the "deadly Amanita," solution taking place without agglutination. Specimens of *Amanita rubescens* dried for several months and then tested are found to have retained their hemolysin unaltered in strength. It is completely destroyed by exposure to a temperature of 70° C. for half an hour, and the extracts so treated contain no Amanita toxin. When passed through a Berkefeld filter and heated to 70° C. they may be given subcutaneously in large doses to both rabbits and guinea-pigs without producing anything more than a transient edema at the site of inoculation. Chemical investigation of *Amanita rubescens* confirms the results of biological experiment. If the aqueous extract of this fungus be evaporated *in vacuo* at 35° C. to a small bulk and then precipitated with ethyl alcohol, the precipitate thus obtained contains the hemolysin. If the alcohol filtrate be evaporated *in vacuo* at 35° C.

to dryness and then taken up in water, it contains no toxin. This fraction may be administered in large quantity to both rabbits and guinea-pigs with practically no effect except a slight subcutaneous edema. The *Amanita rubescens*, therefore, while containing powerful hemolysins, contains no Amanita toxin, i. e., none of the active principle of the "deadly Amanita."

NATURE OF THE RUBESCENS-HEMOLYSIN.

Like the hemolysin found in *Amanita phalloides*, and shown by Abel and Ford[1] to be a glucoside, the hemolysin in the *rubescens* is probably also a glucoside. By the methods already indicated[2] both uranyl acetate and freshly prepared metaphosphoric acid will remove the proteid from both concentrated and fresh extracts of the fungus, leaving the hemolysin unaltered in strength. After removal of the proteid, the solutions containing the still active hemolysins reduce Fehling's solution and ammoniacal silver nitrate directly, but have their reducing property increased by hydrolysis with dilute hydrochloric acid. The extracts ferment actively with brewer's yeast, pointing to the presence of free glucose. After treatment with both metaphosphoric acid and uranyl acetate, the actively hemolytic solutions, like those of the amanita-hemolysin, give no precipitate with phosphotungstic and phosphomolybdic acid, and the precipitate given by tannic acid is soluble in excess. An abundant precipitate is produced on the addition of basic lead acetate and also by cupric acetate. All tests for pentoses, α-naphthol and sulphuric acid, phloroglucin and hydrochloric acid, orcin, ferric chloride and hydrochloric acid have thus far given negative results. The *rubescens* hemolysin is therefore a glucoside, the exact composition of which is still under investigation. The important fact is that we have in this edible fungus powerful hemolysins but no Amanita toxin, the active principle of the "deadly Amanita."

AMANITA SOLITARIA.

This very rare fungus has an especial interest because it contains substances having a peculiar action upon blood corpuscles. It grows late in the summer, usually in the dry sandy soil just at the edge of

[1] *Loc. cit.*

[2] Abel and Ford, *loc. cit.*

the roads. It is a very large Amanita, pure white in color, developing from a universal cup like *Amanita caesaria*. In its early stages this cup forms an egg-shaped mass just projecting above the surface of the ground. As the plant matures the cup bursts and from its center the pileus issues, its surface covered with huge moist scales representing the remains of the cup. In the adult forms both pileus and stalk are covered with these scales, which give a fringed appearance to the entire plant. The scales are easily removed by brushing lightly with the fingers, but are quite viscid and sticky. The general structure of the plant resembles that of *Amanita phalloides*, but it is easily distinguished by its very large size and by its fringed surface. It is considered edible by most mycologists, by Atkinson and others, the danger from its use coming from mistakes in identification. No cases of poisoning due to it are on record.

Aqueous or saline extracts of *Amanita solitaria* are hemolytic, but not to such a degree as are other hemolytic Amanitas. The lysis is preceded by a typical agglutination of the corpuscles, which sink to the bottom of the tube in a densely adherent mass. The agglutination is slow, requiring one to three hours, and after this time a slow solution of the corpuscles takes place, requiring four to five hours. With concentrated extracts of perfectly fresh plants the reaction is more rapid, sometimes being complete at the end of two hours. The action upon corpuscles is thus seen to be closely analogous to that of an immune serum by which the erythrocytes are first agglutinated and then dissolved. When heated to 70° C., for half an hour, the hemolysin is destroyed, and filtered extracts heated to this temperature are innocuous to both rabbits and guinea-pigs. Even when introduced in concentrated solution only a transient edema is produced. Chemical analysis of the fungus confirms these observations. If extracts be evaporated to a small bulk *in vacuo* at 35° C., and then precipitated with ethyl alcohol, the precipitate contains the agglutino-hemolysin. The alcohol filtrate evaporated to dryness and taken up in water contains no Amanita toxin, and may be given to animals in large amounts without harmful results. Proteid may be removed from extracts of the "solitaria" by both the uranyl acetate and metaphosphoric acid methods, the proteid-free solutions retaining their power of agglutinating and dissolving the

erythrocytes. Uranyl acetate serves this purpose far better than does metaphosphoric acid, the latter destroying the hemolysin unless it be quickly neutralized by sodium bicarbonate. The proteid-free solutions reduce Fehling's solution and ammoniacal silver nitrate before boiling with hydrochloric acid, but have this property enhanced by this hydrolysis. No precipitate is given by phosphotungstic acid and the precipitate from tannic acid is soluble in excess. Precipitates are also given by basic lead acetate and by cupric acetate. Tests for pentoses with α-naphthol, orcin, and phloroglucin have thus far been negative.

The substances in *Amanita solitaria* acting upon blood corpuscles are thus seen to be glucosides, but differing markedly from those found in either *Amanita phalloides* or *Amanita rubescens*. It has not been possible to separate the agglutinating from the hemolytic action so that it cannot be stated whether more than one substance comes into play in this phenomenon or a single substance. As in the case of *Amanita rubescens*, the importance of the observation lies in the fact that an edible fungus may contain blood-laking principles but no Amanita toxin.